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Diagnostic and therapeutic use of a voltage-gated ion channel for neurodegenerative disease

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DIAGNOSTIC AND THERAPEUTIC USE OF A VOLTAGE-GATED ION CHANNEL FOR NEURODEGENERATIVE DISEASES

The present invention relates to methods of diagnosing, prognosticating and monitoring the progression of neurodegenerative diseases in a subject. Furthermore, methods of therapy control and screening for modulating agents of neurodegenerative diseases are provided. The invention also discloses pharmaceutical compositions, kits, and recombinant animal models.

Neurodegenerative diseases, in particular Alzheimer's disease (AD), have a strongly debilitating impact on a patient's life. Furthermore, these diseases constitute an enormous health, social and economic burden. Alzheimer's disease is the most common age-related neurodegenerative condition affecting about 10 % of the population over 65 years of age and up to 45 % over age 85 (for a recent review see Vickers et al., *Progress in Neurobiology* 2000, 60: 139-165). Presently this amounts to an estimated 12 million cases in the US, Europe, and Japan. This situation will inevitably worsen with the demographic increase in the number of old people ("aging of the baby boomers") in developed countries. The neuropathological hallmarks that occur in the brains of individuals with Alzheimer's disease are senile plaques, composed of amyloid-β protein, and profound cytoskeletal changes coinciding with the appearance of abnormal filamentous structures and the formation of neurofibrillary tangles. AD is a progressive disease that is associated with early deficits in memory formation and ultimately leads to the complete erosion of higher cognitive function. A characteristic feature of the pathogenesis of AD is the selective vulnerability of particular brain regions and subpopulations of nerve cells to the degenerative process. Specifically, the temporal lobe region and the hippocampus are affected early and more severely during the progression of the disease. On the other hand, neurons within the frontal cortex, occipital cortex, and the cerebellum remain largely intact and are protected from neurodegeneration (Terry et al., *Annals of Neurology* 1981, 10: 184-192).

Currently, there is no cure for AD, nor is there an effective treatment to halt the progression of AD or even to diagnose AD ante-mortem with high probability. Several risk factors have been identified that predispose an individual to develop AD, among them most prominently the epsilon4 allele of apolipoprotein E (ApoE). Although there are rare examples of early-onset AD which have been attributed to genetic defects in the genes for amyloid precursor protein (APP), presenilin-1, and presenilin-2, the prevalent form of late-onset sporadic AD is of hitherto unknown etiologic origin. The late onset and complex pathogenesis of neurodegenerative disorders pose a formidable challenge to the development of therapeutic and diagnostic agents. It is crucial to expand the pool of potential drug targets and diagnostic markers. It is therefore an object of the present invention to provide insight into the pathogenesis of neurological diseases and to provide methods, materials, and animal models which are suited inter alia for the diagnosis and development of a treatment of these diseases. This object has been solved by the features of the independent claims. The subclaims define preferred embodiments of the present invention.

Voltage-gated ion channels play an important role in the nervous system by generating conducted action potentials. Nowadays, ion-conducting membrane channels for cations (sodium, calcium, potassium) and anions (chloride) are described (Lehmann-Horn et al., *Physiological Reviews* 1999, 79: 1317-1358). Transport of ions across the cell membrane leads to a fast transmission of electrical impulses throughout the cell network. Thereby the channel switches between three functionally distinct states: a resting, an active, and an inactive one. Both, the resting and inactive states are nonconducting, and the channel is closed. As the membrane potential increases from less than –60 mV, the channel starts to open its pore (i.e. activation). Influx of ions (e.g. sodium) leads to a further increase of the membrane potential until an action potential is initiated. By closing the pore within 1 millisecond (i.e. fast inactivation) or within seconds to minutes (i.e. slow inactivation), the channel rapidly returns to an inactivated state. The ion conductance is highly selective and efficient

which enables fine tuning of processes such as memory, movement, and cognition (Lehmann-Horn et al., *Physiological Reviews* 1999, 79: 1317-1358). Molecular cloning of voltage-gated ion channels has uncovered a diversity of subtypes and enhanced the understanding about the underlying structure and function, particularly of sodium channels (Noda et al., *Nature* 1986, 322: 826-828; Schaller et al., *Journal of Neuroscience* 1995, 15: 3231-3242; Isom et al., *Neuron* 1994, 12: 1183-1194; Isom et al., *Cell* 1995, 83: 443-445).

Sodium channels exist as tetramers of four identical homologous domains (DI – DIV), each consisting of six transmembrane helices (S1- S6) which form a group around the central ion-conducting pore. A precise three-dimensional structure is still not available (Catterall et al., *Advances in Neurology* 1999, 79: 441-456). A highly glycosylated α-subunit with approximately 260 kDa and two β-subunits (β1 with ~36kDa and β2 with ~33kDa) form a heteromeric complex, whereby the β1-subunit is noncovalently associated and the β2-subunit is covalently attached to the α-subunit via a disulfide bridge. A third β-subunit isoform similar to the β1-subunit, also attached to the α-subunit, has recently been discovered (Morgan et al., *Proceedings National Academy of Science USA* 2000, 97: 2308-2313). The α-subunit appears to be necessary and sufficient for sodium channel functionality. The β-subunit modulates sodium channel function by accelerating activation and inactivation processes by increasing peak current and by altering voltage dependency (Patton et al., *Journal Biological Chemistry* 1994, 269: 17649-17655). β-subunits exhibit an immunoglobulin-like motif with structural similarities to neuronal cell adhesion molecules which may interact with extracellular matrix proteins (Isom et al., *Cell* 1995, 83: 443-445). An important mechanism for modulation of sodium channel properties is the rate of glycosylation and the change in their phosphorylation state. Sodium channels have multiple sites for phosphorylation by protein kinases A and C (PKA and PKC). Phosphorylation of these sites results in slowed inactivation and reduced peak current.

Currently, twelve different human α-subunits genes have been cloned (designated SCN1A to SCN12A) and found to be organized in four conserved transmembrane segments. They are known to be expressed in mammalian brain

and peripheral tissues and they show tissue-specific expression, with individual cell types expressing different complements of sodium channels, e.g. SCN4A is expressed primarily in skeletal muscle, SCN5A and SCN6A in cardiac muscle, whereas SCN1A, 2A, 3A, 8A, 9A, 10A, 11A and 12A are expressed in neurons of the central nervous system. The four brain sodium channels which exhibit major expression in the central nervous system (SCN1A, SCN2A, SCN3A, SCN8A) show high amino acid sequence similarity (90% to 75%). The overall similarities between the voltage-gated sodium channels (i.e. sodium channel gene family) suggest a single evolutionary origin and subsequent α-gene duplication events.

To date, a number of genetic mutations have been identified which affect the function of the above described sodium channels. For example, an underlying cause for generalized epilepsy are mutations in the SCN1A gene (Kearney et al., *Neurosciences* 2001, 2: 307-317). Various periodic paralysis syndromes and hyperexcitability, as found associated with LQT Syndrome, have been linked to mutations in skeletal and cardiac sodium channels (SCN4A, SCN5A) (Lehmann-Horn et al., *Physiological Reviews* 1999, 79: 1317-1358).

Sodium channels are valuable targets for a variety of drugs as local anesthetics, anticonvulsants, antiarrythmics, for the treatment of neuropathic pain, epilepsy, and stroke. Although a number of toxins, drugs, and inorganic cations are used by the pharmaceutical industry as blockers in central nervous system related disorders, and although a number of inhibitors of voltage-gated ion channels are on the market, the therapeutic potential of currently used drugs is not fully exploited. They are of low potency and relatively non-specific. Thus, it is required to find specific drugs for a selective target known to be associated with a specific clinical condition. To date, there are no reports on a relationship between a voltage-gated ion channel and neurodegenerative disorders such as Alzheimer's disease. Such a link, as disclosed in the present invention, offers new ways, inter alia, for the diagnosis and treatment of these disorders.

The first report about the structure and chromosomal location of sodium shannel type 2A (SCN2A) was published in 1992 (denoted as HBA; GenBank Accession No. M94055; X65361; Ahmed et al., *Proc. Natl. Acad. of Sci. USA* 1992, 89: 820-824). A further description of the genomic structure of the SCN2A gene was revealed in 2001 (GenBank Accession No. AF327246; AH010232; GDB ID: 120367; Kasai et al., *Gene* 2001, 264: 113-122). Herein, SCN2A was characterized as a positional candidate gene for the deafness disorder DFNA16, a form of autosomal dominant non-syndromic hearing loss (ADNSHL). Fine mapping studies clearly define the chromosomal location to the map locus 2q23-q24.3. SCN2A covers approximately 120 kb of genomic DNA, harboring 29 exons (54 bp to 1196 bp in size) which encode for a protein of 2005 amino acids. The SCN2A gene is expressed primarily in the central nervous system and in the cochlea. Two alternatively spliced isoforms of SCN2A (exon 6A, exon 6N) were identified, and as a result three mRNA variants were detected, i.e. SCN2A harboring exon 6A, or exon 6N, or none of both. The exon 6A encoding transcript was found to be expressed in human adult brain, and the transcript harboring exon 6N was detected in human fetal brain and lymphocytes. The transcript with deleted exon 6 was found to be expressed in lymphocytes only (Kasai et al., *Gene* 2001, 264: 113-122). In addition to tissue-specific expression of the two alternatively spliced SCN2A isoforms, the SCN2A gene is developmentally regulated. SCN2A type 6A exon is expressed throughout development, with highest levels in rostral brain regions (brainstem, hippocampus, cortex, striatum, midbrain) (Whitaker et al., *Journal of Comparative Neurology* 2000, 422: 123-139; Planells-Cases et al., *Biophysical Journal* 2000, 78: 2878-2891), whereas SCN2A type 6N exon was found to be present only in fetal tissue. The subcellular distribution of SCN2A polypeptides is characterized by location along the axons of neurons, preferentially on unmyelinated projection fibers. This suggests a highly distinct function of the SCN2A channels.

A comparative expression study on the cellular level has been published by Whitaker in 2001 (*Molecular Brain Research* 2001, 88: 37-53). The study compared tissues from normal and from epileptic hippocampus and found

SCN2A to be downregulated in pyramidal cells, whereas other sodium channels, such as SCN3A, were upregulated. Recently, several mutations in the SCN2A gene have been identified (Arg1638His; in DIV, S6) (Kasai et al., *Gene* 2001, 264: 113-122), none of which cosegregate with a pathological phenotype (e.g. increased neuronal excitability, susceptibility to seizures).

An animal model for seizure disorders is the so called Q54-mouse. This mouse expresses a transgene with a gain-of-function mutation in domain DII, S4-S5 of the SCN2A gene (Kearney et al., *Neuroscience* 2001, 102: 307-317) resulting in a profound phenotype despite endogenous SCN2A gene expression. A homozygous SCN2A knock-out mouse (deletion of exon 1 of SCN2A gene) shows severe defects and results in mortality around the time of birth (Planells-Cases et al., *Biophysical Journal* 2000, 78: 2878-2891).

The term "and/or" as used in the present specification and in the claims implies that the phrases before and after this term are to be considered either as alternatives or in combination. For instance, the wording "determination of a level and/or an activity" means that either only a level, or only an activity, or both a level and an activity are determined. The term "level" as used herein is meant to comprise a gage of, or a measure of the amount of, or a concentration of a transcription product, for instance an mRNA, or a translation product, for instance a protein or polypeptide. The term "activity" as used herein shall be understood as a measure for the ability of a transcription product or a translation product to produce a biological effect or a measure for a level of biologically active molecules. The term "activity" also refers to enzymatic activity. The terms "level" and/or "activity" as used herein further refer to gene expression levels or gene activity. Gene expression can be defined as the utilization of the information contained in a gene by transcription and translation leading to the production of a gene product. A gene product comprises either RNA or protein and is the result of expression of a gene. The amount of a gene product can be used to measure how active a gene is. The term "gene" as used in the present specification and in the claims comprises both coding regions (exons) as well as non-coding regions

(e.g. non-coding regulatory elements such as promoters or enhancers, introns, leader and trailer sequences). The term "fragment" as used herein is meant to comprise e.g. an alternatively spliced, or truncated, or otherwise cleaved transcription product or translation product. The term "derivative" as used herein refers to a mutant, or an RNA-edited, or a chemically modified, or otherwise altered transcription product, or to a mutant, or chemically modified, or otherwise altered translation product. For instance, a "derivative" may be generated by processes such as altered phosphorylation, or glycosylation, or lipidation, or by altered signal peptide cleavage or other types of maturation cleavage. These processes may occur post-translationally. The term "modulator" as used in the present invention and in the claims refers to a molecule capable of changing or altering the level and/or the activity of a gene, or a transcription product of a gene, or a translation product of a gene. Preferably, a "modulator" is capable of changing or altering the biological activity of a transcription product or a translation product of a gene. Said modulation, for instance, may be an increase or a decrease in enzyme activity, a change in binding characteristics, or any other change or alteration in the biological, functional, or immunological properties of said translation product of a gene.

In one aspect, the invention features a method of diagnosing or prognosticating a neurodegenerative disease in a subject, or determining whether a subject is at increased risk of developing said disease. The method comprises: determining a level, or an activity, or both said level and said activity of (i) a transcription product of a gene coding for a voltage-gated ion channel, and/or of (ii) a translation product of a gene coding for a voltage-gated ion channel, and/or of (iii) a fragment or derivative of said transcription or translation product in a sample from said subject and comparing said level, and/or said activity to a reference value representing a known disease or health status, thereby diagnosing or prognosticating said neurodegenerative disease in said subject, or determining whether said subject is at increased risk of developing said neurodegenerative disease.

In a further aspect, the invention features a method of monitoring the progression of a neurodegenerative disease in a subject. A level, or an activity, or both said level and said activity, of (i) a transcription product of a gene coding for a voltage-gated ion channel, and/or of (ii) a translation product of a gene coding for a voltage-gated ion channel, and/or of (iii) a fragment or derivative of said transcription or translation product in a sample from said subject is determined. Said level and/or said activity is compared to a reference value representing a known disease or health status. Thereby the progression of said neurodegenerative disease in said subject is monitored.

In still a further aspect, the invention features a method of evaluating a treatment for a neurodegenerative disease, comprising determining a level, or an activity, or both said level and said activity of (i) a transcription product of a gene coding for a voltage-gated ion channel, and/or of (ii) a translation product of a gene coding for a voltage-gated ion channel, and/or of (iii) a fragment or derivative of said transcription or translation product in a sample obtained from a subject being treated for said disease. Said level, or said activity, or both said level and said activity are compared to a reference value representing a known disease or health status, thereby evaluating the treatment for said neurodegenerative disease.

In a preferred embodiment, said subjects suffer from Alzheimer's disease. Further examples of neurodegenerative diseases are Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, Pick's disease, fronto-temporal dementia, progressive nuclear palsy, and corticobasal degeneration.

It is preferred that said voltage-gated ion channel is a member of the sodium channel gene family , particularly SCN2A. The present invention discloses the differential expression and regulation of the SCN2A gene in specific brain regions of Alzheimer's disease patients. Consequently, the SCN2A gene and its corresponding translation products may have a causative role in the regional selective neuronal degeneration typically observed in Alzheimer's disease. Alternatively, SCN2A may confer a neuroprotective function to the

remaining surviving nerve cells. Based on these disclosures, the present invention has utility for the diagnostic evaluation and prognosis as well as for the identification of a predisposition to a neurodegenerative disease, in particular Alzheimer's disease. Furthermore, the present invention provides methods for the diagnostic monitoring of patients undergoing treatment for such a disease.

It is particularly preferred that said sample to be analyzed and determined is selected from the group consisting of a brain tissue or other tissues, organs or body cells. The sample can also consist of cerebrospinal fluid or other body fluids including saliva, urine and blood, serum plasma, or nasal mucosa.

In further preferred embodiments, said reference value is that of a level, or an activity, or both said level and said activity of (i) a transcription product of a gene coding for a voltage-gated ion channel, and/or of (ii) a translation product of a gene coding for a voltage-gated ion channel, and/or of (iii) a fragment or derivative of said transcription or translation product in a sample from a subject not suffering from said neurodegenerative disease.

In preferred embodiments, an increase or decrease in SCN2A mRNA and /or SCN2A protein in a sample cell or tissue from said subject relative to a reference value representing a known health status indicates a diagnosis, or [illegible]s, or increased risk of becoming diseased with a neurodegenerative [illegible]ease, particularly Alzheimer's disease.

[illegible]ed embodiments, measurement of the level of transcription products of a gene coding for a voltage-gated ion channel is performed in a sample [illegible] a subject using a quantitative PCR-analysis with primer combinations to [illegible]ify said gene specific sequences from cDNA obtained by reverse [illegible]ription of RNA extracted from a sample of a subject. A Northern blot [illegible] probes specific for said gene can also be applied. These techniques are [illegible] to those of ordinary skill in the art (see Sambrook and Russell,

Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2000).

Furthermore, the level of a translation product of a gene coding for a voltage-gated ion channel and/or fragment of said translation product, and/or level of activity of said translation product and/or fragment of said translation product, can be detected using an immunoassay, an activity assay and/or binding assay. These assays can measure the amount of binding between said protein molecule and an anti-protein antibody by the use of enzymatic, chromodynamic, radioactive, or luminescent labels which are attached to either the anti-protein antibody or a secondary antibody which binds the anti-protein antibody. In addition, other high affinity ligands may be used. Immunoassays which can be used include e.g. ELISAs, Western blots and other techniques known to those of ordinary skill in the art (see Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1999).

In a preferred embodiment, the level, or the activity, or both said level and said activity of (i) a transcription product of a gene coding for a voltage-gated ion channel, and/or of (ii) a translation product of a gene coding for a voltage-gated ion channel, and/or of (iii) a fragment or derivative of said transcription or translation product in a series of samples taken from said subject over a period of time is compared, in order to monitor the progression of said disease. In further preferred embodiments, said subject receives a treatment prior to one or more of said sample gatherings. In yet another preferred embodiment, said level and/or activity is determined before and after said treatment of said subject.

In another aspect, the invention features a kit for diagnosing or prognosticating Alzheimer's disease in a subject, or determining the propensity or predisposition of a subject to develop Alzheimer's disease, said kit comprising:

(a) at least one reagent which is selected from the group consisting of (i) reagents that selectively detect a transcription product of a gene coding for a voltage-gated ion channel (ii) reagents that selectively detect a translation product of a gene coding for a voltage-gated ion channel; and
(b) instruction for diagnosing, or prognosticating Alzheimer's disease, or determining the propensity or predisposition of a subject to develop Alzheimer's disease by

- detecting a level, or an activity, or both said level and said activity, of said transcription product and/or said translation product of a gene coding for a voltage-gated ion channel, in a sample from said subject; and
- diagnosing or prognosticating Alzheimer's disease, or determining the propensity or predisposition of said subject to develop Alzheimer's disease,

wherein a varied level, or activity, or both said level and said activity, of said transcription product and/or said translation product compared to a reference value representing a known health status; or a level, or activity, or both said level and said activity, of said transcription product and/or said translation product similar or equal to a reference value representing a known disease status, indicates a diagnosis or prognosis of Alzheimer's disease, or an increased propensity or predisposition of developing Alzheimer's disease. The kit, according to the present invention, may be particularly useful for the identification of individuals that are at risk of developing Alzheimer's disease. Consequently, the kit, according to the invention, may serve as a means for targeting identified individuals for early preventive measures or therapeutic intervention prior to disease onset, before irreversible damage in the course of the disease has been inflicted. Furthermore, in preferred embodiments, the kit featured in the invention is useful for monitoring a progression of Alzheimer's disease in a subject, as well as monitoring success or failure of therapeutic treatment for Alzheimer's disease of said subject.

In another aspect, the invention features a method of treating or preventing a neurodegenerative disease, in particular Alzheimer's disease, in a subject

comprising the administration to said subject in a therapeutically or prophylactically effective amount of an agent or agents which directly or indirectly affect a level, or an activity, or both said level and said activity, of (i) a gene coding for a voltage-gated ion channel, and/or (ii) a transcription product of a gene coding for a voltage-gated ion channel, and/or (iii) a translation product of said gene, and/or (iv) a fragment or derivative of (i) to (iii).

In preferred embodiments, the method comprises the application of per se known methods of gene therapy and/or antisense nucleic acid technology to administer said agent or agents. In general, gene therapy includes several approaches: molecular replacement of a mutated gene, addition of a new gene resulting in the synthesis of a therapeutic protein, and modulation of endogenous cellular gene expression by recombinant expression methods or by drugs. Gene-transfer techniques are described in detail (see e.g. Behr, *Acc Chem Res* 1993, 26: 274-278 and Mulligan, *Science* 1993, 260: 926-931; the contents of which are incorporated herein by reference) and include direct gene-transfer techniques such as mechanical microinjection of DNA into a cell as well as indirect techniques employing biological vectors (like recombinant viruses, especially retroviruses) or model liposomes, or techniques based on transfection with DNA coprecipitation with polycations, cell membrane pertubation by chemical (solvents, detergents, polymers, enzymes) or physical means (mechanic, osmotic, thermic, electric shocks). The postnatal gene transfer into the central nervous system has been described in detail (see e.g. Wolff, *Curr Opin Neurobiol* 1993, 3: 743-748).

In particular, the invention features a method of treating or preventing a neurodegenerative disease by means of antisense nucleic acid therapy, i.e. the down-regulation of an inappropriately expressed or defective gene by the introduction of antisense nucleic acids or derivatives thereof into certain critical cells (see e.g. Gillespie, *DN&P* 1992, 5: 389-395; Agrawal and Akhtar, *Trends Biotechnol* 1995, 13: 197-199; Crooke, *Biotechnology* 1992, 10: 882-6; the contents of which are incorporated herein by reference). Apart from hybridization strategies, the application of ribozymes, i.e. RNA molecules that

act as enzymes, destroying RNA that carries the message of disease has also been described (see e.g. Barinaga, *Science* 1993, 262: 1512-1514; the contents of which are incorporated herein by reference). In preferred embodiments, the subject to be treated is a human, and therapeutic antisense nucleic acids or derivatives thereof are directed against a human voltage-gated ion channel, particularly SCN2A . It is preferred that cells of the central nervous system, preferably the brain, of a subject are treated in such a way. Cell penetration can be performed by known strategies such as coupling of antisense nucleic acids and derivatives thereof to carrier particles, or the above described techniques. Strategies for administering targeted therapeutic oligodeoxynucleotides are known to those of skill in the art (see e.g. Wickstrom, *Trends Biotechnol* 1992, 10: 281-287; the contents of which are incorporated herein by reference). In some cases, delivery can be performed by mere topical application. Further approaches are directed to intracellular expression of antisense RNA. In this strategy, cells are transformed *ex vivo* with a recombinant gene that directs the synthesis of an RNA that is complementary to a region of target nucleic acid. Therapeutical use of intracellularly expressed antisense RNA is procedurally similar to gene therapy.

In further preferred embodiments, the method comprises grafting donor cells into the central nervous system, preferably the brain, of said subject, or donor cells preferably treated so as to minimize or reduce graft rejection, wherein said donor cells are genetically modified by insertion of at least one transgene encoding said agent or agents. Said transgene might be carried by a viral vector, in particular a retroviral vector. The transgene can be inserted into the donor cells by a nonviral physical transfection of DNA encoding a transgene, in particular by microinjection. Insertion of the transgene can also be performed by electroporation, chemically mediated transfection, in particular calcium phosphate transfection, liposomal mediated transfection.

In preferred embodiments, said agent is a therapeutic protein which can be administered to said subject, preferably a human, by a process comprising

introducing subject cells into said subject, said subject cells having been treated *in vitro* to insert a DNA segment encoding said therapeutic protein, said subject cells expressing *in vivo* in said subject a therapeutically effective amount of said therapeutic protein. Said DNA segment can be inserted into said cells *in vitro* by a viral vector, in particular a retroviral vector.

Methods of treatment, according to the present invention, comprise the application of therapeutic cloning, transplantation, and stem cell therapy using embryonic stem cells or embryonic germ cells and neuronal adult stem cells, combined with any of the previously described cell- and gene therapeutic methods. Stem cells may be totipotent or pluripotent. They may also be organ-specific. Strategies for repairing diseased and/or damaged brain cells or tissue comprise (i) taking donor cells from an adult tissue. Nuclei of those cells are transplanted into unfertilized egg cells from which the genetic material has been removed. Embryonic stem cells are isolated from the blastocyst stage of the cells which underwent somatic cell nuclear transfer. Use of differentiation factors then leads to a directed development of the stem cells to specialized cell types, preferably neuronal cells (Lanza et al., *Nature Medicine* 1999, 9: 975-977), or (ii) purifying adult stem cells, isolated from the central nervous system, or from bone marrow (mesenchymal stem cells), for in vitro expansion and subsequent grafting and transplantation, or (iii) directly inducing endogenous neural stem cells to proliferate, migrate, and differentiate into functional neurons (Peterson DA, *Curr. Opin. Pharmacol.* 2002, 2: 34-42). Adult neural stem cells are of great potential for repairing damaged or diseased brain tissues, as the germinal centers of the adult brain are free of neuronal damage or dysfunction (Colman A, *Drug Discovery World* 2001, 7: 66-71).

In preferred embodiments, the subject for treatment or prevention, according to the present invention, can be a human, an experimental animal, e.g. a mouse or a rat, a domestic animal, or a non-human primate. The experimental animal can be an animal model for a neurodegenerative disorder, e.g. a

transgenic mouse and/or a knock-out mouse with an Alzheimer's-type neuropathology.

In a further aspect, the invention features a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) a gene coding for a voltage-gated ion channel, and/or (ii) a transcription product of a gene coding for a voltage-gated ion channel and/or (iii) a translation product of a gene coding for a voltage-gated ion channel, and/or (iv) a fragment or derivative of (i) to (iii).

In an additional aspect, the invention features a pharmaceutical composition comprising said modulator and preferably a pharmaceutical carrier. Said carrier refers to a diluent, adjuvant, excipient, or vehicle with which the modulator is administered.

In a further aspect, the invention features a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) a gene coding for a voltage-gated ion channel, and/or (ii) a transcription product of a gene coding for a voltage-gated ion channel, and/or (iii) a translation product of a gene coding for a voltage-gated ion channel, and/or (iv) a fragment or derivative of (i) to (iii) for use in a pharmaceutical composition.

In another aspect, the invention provides for the use of a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) a gene coding for a voltage-gated ion channel, and/or (ii) a transcription product of a gene coding for a voltage-gated ion channel and/or (iii) a translation product of a gene coding for a voltage-gated ion channel, and/or (iv) a fragment or derivative of (i) to (iii) for a preparation of a medicament for treating or preventing a neurodegenerative disease, in particular Alzheimer's disease.

In one aspect, the present invention also provides a kit comprising one or more containers filled with a therapeutically or prophylactically effective amount of said pharmaceutical composition.

In a further aspect, the invention features a recombinant, non-human animal comprising a non-native gene sequence coding for a voltage-gated ion channel, or a fragment thereof, or a derivative thereof. The generation of said recombinant, non-human animal comprises (i) providing a gene targeting construct containing said gene sequence and a selectable marker sequence, and (ii) introducing said targeting construct into a stem cell of a non-human animal, and (iii) introducing said non-human animal stem cell into a non-human embryo, and (iv) transplanting said embryo into a pseudopregnant non-human animal, and (v) allowing said embryo to develop to term, and (vi) identifying a genetically altered non-human animal whose genome comprises a modification of said gene sequence in both alleles, and (vii) breeding the genetically altered non-human animal of step (vi) to obtain a genetically altered non-human animal whose genome comprises a modification of said endogenous gene, wherein said gene is mis-expressed, or under-expressed, or over-expressed, and wherein said disruption or alteration results in said non-human animal exhibiting a predisposition to developing symptoms of neuropathology similar to a neurodegenerative disease, in particular Alzheimer's disease. Strategies and techniques for the generation and construction of such an animal are known to those of ordinary skill in the art (see e.g. Capecchi, *Science* 1989, 244: 1288-1292 and Hogan et al., 1994, *Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; the contents of the foregoing are incorporated herein by reference). It is preferred to make use of such a recombinant non-human animal as an animal model for investigating neurodegenerative diseases, in particular Alzheimer's disease. In preferred embodiments, said recombinant, non-human animal comprises a non-native gene sequence coding for a member of the sodium channel gene family, in particular SCN2A, or a fragment thereof.

In another aspect, the invention features an assay for screening for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases and disorders of one or more substances selected from the group consisting of (i) a gene coding for a voltage-gated ion channel, and/or (ii) a transcription product of a gene coding for a voltage-gated ion channel, and/or (iii) a translation product of a gene coding for a voltage-gated ion channel, and/or (iv) a fragment or derivative of (i) to (iii). This screening method comprises (a) contacting a cell with a test compound, and (b) measuring the activity, or the level, or both the activity and the level of one or more substances recited in (i) to (iv), and (c) measuring the activity, or the level, or both the activity and the level of said substances in a control cell not contacted with said test compound, and (d) comparing the levels of the substance in the cells of step (b) and (c), wherein an alteration in the activity and/or level of said substances in the contacted cells indicates that the test compound is a modulator of said diseases and disorders.

In one further aspect, the invention features a screening assay for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases and disorders of one or more substances selected from the group consisting of (i) a gene coding for a voltage-gated ion channel, and/or (ii) a transcription product of a gene coding for a voltage-gated ion channel, and/or (iii) a translation product of a gene coding for a voltage-gated ion channel, and/or (iv) a fragment or derivative of (i) to (iii), comprising (a) administering a test compound to a test animal which is predisposed to developing or has already developed a neurodegenerative disease or related diseases or disorders, and (b) measuring the activity and/or level of one or more substances recited in (i) to (iv), and (c) measuring the activity and/or level of said substances in a matched control animal which is equally predisposed to developing or has already developed said diseases and to which animal no such test compound has been administered, and (d) comparing the activity and/or level of the substance in the animals of step (b) and (c), wherein an alteration in the activity and/or level of substances in the

test animal indicates that the test compound is a modulator of said diseases and disorders.

In a preferred embodiment, said test animal and/or said control animal is a recombinant, non-human animal which expresses a voltage-gated ion channel, or a fragment thereof, or a derivative thereof, under the control of a transcriptional regulatory element which is not the native voltage-gated ion channel gene transcriptional control regulatory element.

In another embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a modulator of neurodegenerative diseases by a method of the aforementioned screening assays and (ii) admixing the modulator with a pharmaceutical carrier. However, said modulator may also be identifiable by other types of screening assays.

In another aspect, the present invention provides for an assay for testing a compound, preferably for screening a plurality of compounds, for inhibition of binding between a ligand and a voltage-gated ion channel, or a fragment or derivative thereof. Said screening assay comprises the steps of (i) adding a liquid suspension of said voltage-gated ion channel, or a fragment or derivative thereof, to a plurality of containers, and (ii) adding a compound or a plurality of compounds to be screened for said inhibition to said plurality of containers, and (iii) adding fluorescently labelled ligand to said containers, and (iv) incubating said voltage-gated ion channel, or said fragment or derivative thereof, and said compound or plurality of compounds, and said fluorescently labelled ligand, and (v) measuring the amounts of fluorescence associated with said voltage-gated ion channel, or with said fragment or derivative thereof, and (vi) determining the degree of inhibition by one or more of said compounds of binding of said ligand to said voltage-gated ion channel, or said fragment or derivative thereof. Instead of utilizing a fluorescently labelled ligand, it might in some aspects be preferred to use any other detectable label known to the person skilled in the art, e.g. radioactive

labels, and detect it accordingly. Said method may be useful for the identification of novel compounds as well as for evaluating compounds which have been improved or otherwise optimized in their ability to inhibit the binding of a ligand to a gene product of a gene coding for a voltage-gated ion channel, or a fragment or derivative thereof. In one further embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a compound as an inhibitor of binding between a ligand and a gene product of a gene coding for a voltage-gated ion channel by the aforementioned inhibitory binding assay and (ii) admixing the compound with a pharmaceutical carrier. However, said compound may also be identifiable by other types of screening assays.

In another aspect, the invention features an assay for testing a compound, preferably for screening a plurality of compounds to determine the degree of binding of said compounds to a voltage-gated ion channel, or to a fragment or derivative thereof. Said screening assay comprises (i) adding a liquid suspension of said voltage-gated ion channel, or a fragment or derivative thereof, to a plurality of containers, and (ii) adding a fluorescently labelled compound or a plurality of fluorescently labelled compounds to be screened for said binding to said plurality of containers, and (iii) incubating said voltage-gated ion channel, or said fragment or derivative thereof, and said fluorescently labelled compound or fluorescently labelled compounds, and (iv) measuring the amounts of fluorescence associated with said voltage-gated ion channel, or with said fragment or derivative thereof, and (v) determining the degree of binding by one or more of said compounds to said voltage-gated ion channel, or said fragment or derivative thereof. In this type of assay it might be preferred to use a fluorescent label. However, any other type of detectable label might also be employed. Said method may be useful for the identification of novel compounds as well as for evaluating compounds which have been improved or otherwise optimized in their ability to bind to a voltage-gated ion channel gene product.

In one further embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a compound as a binder to a gene product of a gene coding for a voltage-gated ion channel by the aforementioned binding assays and (ii) admixing the compound with a pharmaceutical carrier. However, said compound may also be identifiable by other types of screening assays.

In another embodiment, the present invention provides for a medicament obtainable by any of the methods according to the herein claimed screening assays. In one further embodiment, the instant invention provides for a medicament obtained by any of the methods according to the herein claimed screening assays.

In all types of assays disclosed herein it is preferred to study a member of the sodium channel gene family. It is particularly preferred to conduct screening assays with the voltage-gated ion channel SCN2A.

The present invention features an antibody which is specifically immunoreactive with an immunogen, wherein said immunogen is a translation product of the SCN2A gene or a fragment thereof. The immunogen may comprise immunogenic or antigenic epitopes or portions of a translation product of said gene, wherein said immunogenic or antigenic portion of a translation product is a polypeptide, and wherein said polypeptide elicits an antibody response in an animal, and wherein said polypeptide is immunospecifically bound by said antibody. Methods for generating antibodies are well known in the art (see Harlow et al., *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). The term "antibody", as employed in the present invention, encompasses all forms of antibodies known in the art, such as polyclonal, monoclonal, chimeric, recombinatorial, or single chain antibodies, as well as fragments thereof. Antibodies of the present invention are useful, for instance, in a variety of diagnostic and therapeutic methods involving detecting translation products of the SCN2A gene.

In a preferred embodiment of the present invention, said antibodies can be used for detecting the pathological state of a cell in a sample from a subject, comprising immunocytochemical staining of said cell with said antibody, wherein an altered degree of staining, or an altered staining pattern in said cell compared to a cell representing a known health status indicates a pathological state of said cell. Preferably, the pathological state relates to a neurodegenerative disease, in particular to Alzheimer's disease. Immunocytochemical staining of a cell can be carried out by a number of different experimental methods well known in the art. It might be preferred, however, to apply an automated method for the detection of antibody binding, wherein the determination of the degree of staining of a cell, or the determination of the cellular or subcellular staining pattern of a cell, or the topological distribution of an antigen on the cell surface or among organelles and other subcellular structures within the cell, are carried out according to the method described by Schubert (US patent 6150173).

Other features and advantages of the invention will be apparent form the following description of figures and examples.

Figure 1 depicts the brain regions with selective vulnerability to neuronal loss and degeneration in Alzheimer's disease. Primarily, neurons within the inferior temporal lobe, the entorhinal cortex, the hippocampus, and the amygdala are subject to degenerative processes in Alzheimer's disease (Terry et al., *Annals of Neurology* 1981, 10:184-192). These brain regions are mostly involved in the processing of learning and memory functions. In contrast, neurons within the frontal cortex, the occipital cortex, and the cerebellum remain largely intact and preserved from neurodegenerative processes in Alzheimer's disease. Brain tissues from the frontal cortex (F) and the temporal cortex (T) of Alzheimer's disease patients and healthy, age-matched control individuals were used for the herein disclosed examples. For illustrative purposes, the image of a normal healthy brain was taken from a publication by Strange

(*Brain Biochemistry and Brain Disorders*, Oxford University Press, Oxford, 1992, p.4).

Figure 2 discloses the initial identification of the differential expression of SCN2A in a suppressive subtractive hybridization screen. The figure shows a clipping of a large-scale dot blot hybridization experiment. Individual cDNA clones from a temporally subtracted library were arrayed onto a nylon membrane and hybridized with probes enriched for genes expressed in the frontal cortex (F) and the temporal cortex (T) of an Alzheimer's disease patient. Ia) clone T16-F11; Ib) clone T16-G11; Ic) clone T16-H11; SCN2A ; IIa) clone T16-F12; IIb) clone T16-G12; IIc) clone T16-H12. Note the significant increase in intensity of the hybridization signal for SCN2A in panel (F) (see arrow head) as compared to the signal in panel (T).

Figure 3 illustrates the verification of the differential expression of SCN2A by quantitative RT-PCR analysis. Quantification of RT-PCR products from RNA samples collected from the frontal cortex (F) and temporal cortex (T) of Alzheimer's disease patients (Fig 3a) and of healthy, age-matched control individuals (Fig 3b) was performed by the LightCycler™ rapid thermal cycling technique. The data were normalized to the combined average values of a set of standard genes which showed no significant differences in their gene expression levels. Said set of standard genes consisted of genes for the ribosomal protein S9, the transferrin receptor, GAPDH, and beta-actin. The figure depicts the kinetics of amplification by plotting the cycle number against the amount of amplified material as measured by its fluorescence. Note that the amplification kinetics of SCN2A cDNA from both the frontal and temporal cortices of a normal control individual during the exponential phase of the reaction overlap (Fig 3b, arrowheads), whereas in Alzheimer's disease (Fig 3a) there is a significant shift of the curve for the sample derived from frontal cortex, indicating an up-regulation of SCN2A mRNA expression in frontal cortex in comparison to temporal cortex.

Figure 4 depicts SEQ ID NO: 1, the nucleotide sequence of the 272 bp SCN2A cDNA fragment, identified and obtained by suppressive subtractive hybridization cloning.

Figure 5 charts the schematic alignment of SEQ ID NO: 1, the SCN2A cDNA fragment, to the nucleotide sequence of the α-subunit of the voltage-gated sodium channel type II (GenBank accession number AF327246). The thick bar represents the SCN2A mRNA, thin bars represent the 3' and 5' untranslated regions (UTR), respectively. The SCN2A cDNA fragment is located within the 3'UTR and is identical to a part of exon 27 of the 8292 bp full-length SCN2A cDNA.

Figure 6 outlines the sequence alignment of SEQ ID NO: 1, the 272 bp SCN2A cDNA fragment, with the nucleotide sequence of the α-subunit voltage-gated sodium channel type II cDNA (GenBank accession number AF327246).

Table 1 lists the gene expression levels in the frontal cortex relative to the temporal cortex for the SCN2A gene in six Alzheimer's disease patients (2.17-3.16Δ fold) and six healthy, age-matched control individuals (0.52-1.37Δ fold).

EXAMPLE I:

(i) Brain tissue dissection from patients with Alzheimer's disease:

Brain tissues from Alzheimer's disease patients and age-matched control subjects were collected within 6 hours post-mortem and immediately frozen on dry ice. Sample sections from each tissue were fixed in paraformaldehyde for histopathological confirmation of the diagnosis. Brain areas for differential expression analysis were identified (see Fig. 1) and stored at –80 °C until RNA extractions were performed.

(ii) Isolation of total mRNA:

Total RNA was extracted from post-mortem brain tissue by using the RNeasy™ kit (Qiagen) according to the manufacturer's protocol. The quality of the prepared RNA was determined by formaldehyde agarose gel electrophoresis and Northern blotting according to standard procedures (Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2000). The mRNA was isolated from the total RNA preparation using the Quickprep Micro™ mRNA Purification Kit (Pharmacia Biotech) with yields between 1 and 5 %.

(iii) cDNA synthesis and identification of differentially expressed genes by suppressive subtractive hybridization:

This technique compares two populations of mRNA and provides clones of genes that are expressed in one population but not in the other. The applied technique was described in detail by Diatchenko et al. (*Proc. Natl. Acad. Sci. USA* 1996, 93: 6025-30) In the present invention, mRNA populations from post-mortem brain tissues from Alzheimer's disease patients were compared. Specifically, mRNA of the frontal cortex was subtracted from mRNA of the inferior temporal cortex. The necessary reagents were taken from the PCR-Select™ cDNA subtraction kit (Clontech), and all steps were performed as described in the manufacturer's protocol. Specifically, 2µg mRNA each were used for first-strand and second-strand cDNA synthesis. After RsaI-digestion and adaptor ligation hybridization of tester and driver was performed for 8 hours (first hybridization) and 15 hours (second hybridization) at 68 °C. Two PCR steps were performed to amplify differentially expressed genes (first PCR: 27 cycles of 94 °C and 30 sec, 66 °C and 30 sec, and 72 °C and 1.5 min; nested PCR: 12 cycles of 94 °C and 30 sec, 66 °C and 30 sec, and 72 °C and 1.5 min) using adaptor specific primers (included in the subtraction kit) and 50x Advantage Polymerase Mix (Clontech). Efficiencies of RsaI-digestions, adaptor ligations and subtractive hybridizations were checked as recommended in the kit. Subtracted cDNAs were inserted into the pCR® vector and transformed into *E.coli* INVαF' cells (Invitrogen).

To isolate individual cDNAs of the subtracted library, single bacterial transformants were incubated in 100 µl LB (with 50 µg/ml ampicillin) at 37 °C

for at least 4 hours. Inserts were PCR amplified (95 °C and 30 sec, 68 °C and 3 min for 30 cycles) in a volume of 20 µl containing 10 mM Tris-HCl pH 9.0, 1.5 mM $MgCl_2$, 50 mM KCl, 200 µM dNTP, 0.5 µM adaptor specific primers (included in the subtraction kit), 1.5 Units Taq polymerase (Pharmacia Biotech), and 1µl of bacterial culture.

1.5 µl of a mixture containing 3 µl PCR amplified inserts and 2 µl 0.3 N NaOH/15 % Ficoll were spotted onto a positively charged nylon membrane (Roche). In this way, hundreds of spots were arrayed on duplicate filters for subsequent hybridization analysis. The differential screening step consisted of hybridizations of the subtracted library with itself to minimize background (Wang and Brown, *Proc. Natl. Acad. Sci. USA* 1991, 88: 11505-9). The probes were generated from the nested PCR product of the subtraction following the instructions of the Clontech subtraction kit. Labeling with digoxigenin was performed with the DIG DNA Labeling Kit (Roche). Hybridizations were carried out overnight in DIG Easy HYB (Roche) at 43 °C. The filters were washed twice in 2 x SSC / 0.5 % SDS at 68 °C for 15 min and twice in 0.1 x SSC / 0.5 % SDS at 68 °C for 15 min, and subjected to detection using anti-DIG-AP conjugates and CDP-Star™ as chemiluminescent substrate according to the instructions of the DIG DNA Detection Kit (Roche). Blots were exposed to Kodak Biomax MR chemiluminescent film at room temperature for several minutes. The nucleotide sequences of clones of interest were obtained using methods well known to those skilled in the art. For nucleotide sequence analyses and homology searches, computer algorithms of the University of Wisconsin Genetics Computer Group (GCG) in conjunction with publicly available nucleotide and peptide sequence information (Genbank and EMBL databases) were employed. The results of one such subtractive hybridization experiment for the SCN2A gene are shown in Fig. 2.

(iv) <u>Confirmation of differential expression by quantitative RT-PCR:</u>

Positive corroboration of differential expression of the SCN2A gene was performed using the LightCycler™ technology (Roche). This technique features rapid thermal cyling for the polymerase chain reaction as well as real-time measurement of fluorescent signals during amplification and

therefore allows for highly accurate quantification of RT-PCR products by using a kinetic, rather than an endpoint approach. The ratio of SCN2A cDNA from the temporal cortex and frontal cortex was determined (relative quantification).

First, a standard curve was generated to determine the efficiency of the PCR with specific primers for SCN2A (5'- TGCAGCAAACAAGGAAGAGCT -3' and 5'-CGGGCTTTTCATCATTGAGTG 3'). PCR amplification (95 °C and 1 sec, 56 °C and 5 sec, and 72 °C and 5 sec) was performed in a volume of 20 µl containing Lightcycler-DNA Master SYBR Green ready-to-use mix (contains Taq DNA polymerase, reaction buffer, dNTP mix with dUTP instead of dTTP, SYBR Green I dye, and 1 mM $MgCl_2$, Roche), additional 3 mM $MgCl_2$, 0,5 µM primers, 0,16 µl TaqStart® antibody (Clontech), and 1 µl of a cDNA dilution series (40, 20, 10, 5, and 1 ng human total brain cDNA, Clontech). Melting curve analysis revealed a single peak at approximately 83°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band of the expected size (74 bp).

In an analogous manner, the PCR protocol was applied to determine the PCR efficiency of a set of reference genes which were selected as a reference standard for quantification. In the present invention, the mean value of five such reference genes was determined: (1) cyclophilin B, using the specific [illegible] 5'-ACTGAAGCACTACGGGCCTG-3' and 5'-AGCCGTTGGTGTCTT-TGCC-3' except for $MgCl_2$ (an additional 1 mM was added instead of 3 mM). [illegible] curve analysis revealed a single peak at approximately 87 °C with no [illegible] primer dimers. Agarose gel analysis of the PCR product showed one [illegible] band of the expected size (62 bp). (2) Ribosomal protein S9 (RPS9), [illegible] the specific primers 5'-GGTCAAATTTACCCTGGCCA-3' and 5'-TCTCATCAAGCGTCAGCAGTTC-3' (exception: additional 1 mM $MgCl_2$ was [illegible] instead of 3 mM). Melting curve analysis revealed a single peak at [illegible]ximately 85°C with no visible primer dimers. Agarose gel analysis of the [illegible] product showed one single band with the expected size (62 bp). (3) [illegible]tin, using the specific primers 5'- TGGAACGGTGAAGGTGACA-3' and

5'- GGCAAGGGACTTCCTGTAA-3'. Melting curve analysis revealed a single peak at approximately 87°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (142 bp). (4) GAPDH, using the specific primers 5'-CGTCATGGGTGTGAACCATG-3' and 5'-GCTAAGCAGTTGGTGGTGCAG-3'. Melting curve analysis revealed a single peak at approximately 83°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (81 bp). (5) Transferrin receptor TRR, using the specific primers 5'- GTCGCTGGTCAGTTCGTGATT-3' and 5'-AGCAGTTGGCTGTTGTACCTCTC-3'. Melting curve analysis revealed a single peak at approximately 83°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (80 bp).

In a first step, the logarithm of the cDNA concentration was plotted against the threshold cycle number C_t for SCN2A and the five reference standard genes. The slopes and the intercepts of the standard curves (i.e. linear regressions) were calculated for all genes. In a second step, cDNA from temporal cortex and frontal cortex was analyzed in parallel and normalized to cyclophilin B. The C_t values were measured and converted to ng total brain cDNA using the corresponding standard curves:

$$10 \wedge ((C_t \text{ value} - \text{intercept}) / \text{slope}) \qquad [\text{ng total brain cDNA}]$$

The values of temporal and frontal cortex SCN2A cDNAs were normalized to cyclophilin B, and the ratio was calculated using the following formula:

$$\text{Ratio} = \frac{\text{SCN2A temporal [ng]} / \text{cyclophilin B temporal [ng]}}{\text{SCN2A frontal [ng]} / \text{cyclophilin B frontal [ng]}}$$

In a third step, the set of reference standard genes was analyzed in parallel to determine the mean average value of the temporal to frontal ratios of

expression levels of the reference standard genes for each individual brain sample. As cyclophilin B was analyzed in step 2 and step 3, and the ratio from one gene to another gene remained constant in different runs, it was possible to normalize SCN2A to the mean average value of the set of reference standard genes instead of normalizing to one single gene alone. The calculation was performed by dividing the ratio shown above by the deviation of cyclophilin B from the mean value of all housekeeping genes.

The results of one such quantitative RT-PCR analysis for the SCN2A gene are shown in Fig. 3.

CLAIMS

1. A method of diagnosing or prognostication a neurodegenerative disease in a subject, or determining whether a subject is at increased risk of developing said disease, comprising:

   determining a level and/or an activity of

   (i) a transcription product of a gene coding for a voltage-gated ion channel, and/or

   (ii) a translation product of a gene coding for a voltage-gated ion channel and/or

   (iii) a fragment or derivative of said transcription or translation product,

   in a sample from said subject and comparing said level and/or said activity to a reference value representing a known disease or health status, thereby diagnosing or prognosticating said neurodegenerative disease in said subject, or determining whether said subject is at increased risk of developing said neurodegenerative disease.

2. A method of monitoring the progression of a neurodegenerative disease in a subject, comprising:

   determining a level and/or an activity of

   (i) a transcription product of a gene coding for a voltage-gated ion channel, and/or

   (ii) a translation product of a gene coding for a voltage-gated ion channel, and/or

   (iii) a fragment or derivative of said transcription or translation product,

   in a sample from said subject and comparing said level and/or said activity to a reference value representing a known disease or health status, thereby monitoring the progression of said neurodegenerative disease in said subject.

3. A method of evaluating a treatment for a neurodegenerative disease, comprising:

   determining a level and/or an activity of

   (i) a transcription product of a gene coding for a voltage-gated ion channel, and/or

   (ii) a translation product of a gene coding for a voltage-gated ion channel, and/or

   (iii) a fragment or derivative of said transcription or translation product,

   in a sample from a subject being treated for said disease and comparing said level and/or said activity to a reference value representing a known disease or health status, thereby evaluating said treatment for said neurodegenerative disease.

4. The method according to any of claims 1 to 3 wherein said neurodegenerative disease is Alzheimer's disease.

5. The method according to any of claims 1 to 4 wherein said voltage-gated ion channel is a member of the sodium channel gene family, in particular SCN2A.

6. The method according to any of claims 1 to 5 wherein said sample is a cell, or a tissue, or an organ, or a body fluid, in particular cerebrospinal fluid or blood.

7. The method according to any of claims 1 to 6 wherein said reference value is that of a level and/or an activity of

   (i) a transcription product of a gene coding for a voltage-gated ion channel, and/or

   (ii) a translation product of a gene coding for a voltage-gated ion channel, and/or

   (iii) a fragment or derivative of said transcription or translation product,

in a sample from a subject not suffering from said neurodegenerative disease.

8. The method according to any of claims 1 to 7 wherein an increase or decrease in SCN2A mRNA and/or SCN2A protein in a cell, or tissue, or body fluid from said subject relative to a reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

9. The method according to any of claims 1 to 8, further comprising comparing a level and/or an activity of
   (i) a transcription product of a gene coding for a voltage-gated ion channel, and/or
   (ii) a translation product of a gene coding for a voltage-gated ion channel, and/or
   (iii) a fragment or derivative of said transcription or translation product

   in a series of samples taken from said subject over a period of time.

10. The method according to claim 9 wherein said subject receives a treatment prior to one or more of said sample gatherings.

11. The method according to claim 10 wherein said level and/or activity is determined before and after said treatment of said subject.

12. A kit for diagnosing or prognosticating Alzheimer's disease in a subject, or determining the propensity or predisposition of a subject to develop Alzheimer's disease, said kit comprising:
   (a) at least one reagent which is selected from the group consisting of
   (I) reagents that selectively detect a transcription product of a gene coding for a voltage-gated ion channel (ii) reagents that selectively detect a translation product of a gene coding for a voltage-gated ion channel and

(b) instruction for diagnosing, or prognosticating Alzheimer's disease, or determining the propensity or predisposition of a subject to develop Alzheimer's disease by

- detecting a level, or an activity, or both said level and said activity, of said transcription product and/or said translation product of a gene coding for a voltage-gated ion channel, in a sample from said subject; and
- diagnosing or prognosticating Alzheimer's disease, or determining the propensity or predisposition of said subject to develop Alzheimer's disease,

wherein a varied level, or activity, or both said level and said activity, of said transcription product and/or said translation product compared to a reference value representing a known health status;

or a level, or activity, or both said level and said activity, of said transcription product and/or said translation product similar or equal to a reference value representing a known disease status indicates a diagnosis or prognosis of Alzheimer's disease, or an increased propensity or predisposition of developing Alzheimer's disease.

13. A method of treating or preventing a neurodegenerative disease, in particular Alzheimer's disease, in a subject comprising administering to said subject in a therapeutically or prophylactically effective amount an agent or agents which directly or indirectly affect an activity and/or a level of (i) a gene coding for a voltage-gated ion channel, and/or (ii) a transcription product of a gene coding for a voltage-gated ion channel, and/or (iii) a translation product of a gene coding for a voltage-gated ion channel, and/or (iv) a fragment or derivative of (i) to (iii).

14. A modulator of an activity and/or of a level of at least one substance which is selected from the group consisting of (i) a gene coding for a voltage-gated ion channel and/or (ii) a transcription product of a gene coding for a voltage-gated ion channel and/or (iii) a translation product of a

gene coding for a voltage-gated ion channel, and/or (iv) a fragment or derivative of (i) to (iii).

15. Use of a modulator of an activity and/or of a level of at least one substance which is selected from the group consisting of (i) a gene coding for a voltage-gated ion channel, and/or (ii) a transcription product of a gene coding for a voltage-gated ion channel, and/or (iii) a translation product of a gene coding for a voltage-gated ion channel, and/or (iv) a fragment or derivative of (i) to (iii) for a preparation of a medicament for treating or preventing a neurodegenerative disease, in particular Alzheimer's disease.

16. A recombinant, non-human animal comprising a non-native gene sequence coding for a voltage-gated ion channel or a fragment thereof, or a derivative thereof, said animal being obtainable by:

(i) providing a gene targeting construct comprising said gene sequence and a selectable marker sequence, and

(ii) introducing said targeting construct into a stem cell of a non-human animal, and

(iii) introducing said non-human animal stem cell into a non-human embryo, and

(iv) transplanting said embryo into a pseudopregnant non-human animal, and

(v) allowing said embryo to develop to term, and

(vi) identifying a genetically altered non-human animal whose genome comprises a modification of said gene sequence in both alleles, and

(vii) breeding the genetically altered non-human animal of step (vi) to obtain a genetically altered non-human animal whose genome comprises a modification of said endogenous gene, wherein said disruption results in said non-human animal exhibiting a predisposition to developing a neurodegenerative disease or related diseases or disorders.

17. The animal according to claim 16 wherein said voltage-gated ion channel is a member of the sodium channel gene family, in particular SCN2A.

18. An assay for screening for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases or disorders of one or more substances selected from the group consisting of

(i) a gene coding for a voltage-gated ion channel, and/or

(ii) a transcription product of a gene coding for a voltage-gated ion channel, and/or

(iii) a translation product of a gene coding for a voltage-gated ion channel, and/or

(iv) a fragment or derivative of (i) to (iii), said method comprising:

(a) contacting a cell with a test compound;

(b) measuring the activity and/or level of one or more substances recited in (i) to (iv);

(c) measuring the activity and/or level of one or more substances recited in (i) to (iv) in a control cell not contacted with said test compound; and

(d) comparing the levels and/or activities of the substance in the cells of step (b) and (c), wherein an alteration in the activity and/or level of substances in the contacted cells indicates that the test compound is a modulator of said diseases or disorders.

19. Use of an antibody specifically immunoreactive with an immunogen, wherein said immunogen is a translation product of the SCN2A gene, or a fragment thereof, for detecting the pathological state of a cell in a sample from a subject, comprising immunocytochemical staining of said cell with said antibody, wherein an altered degree of staining, or an altered staining pattern in said cell compared to a cell representing a known health status indicates a pathological state of said cell, and wherein said pathological state relates to a neurodegenerative disease, preferably Alzheimer's disease.

SUMMARY

The present invention discloses the differential expression of the voltage-gated ion channel SCN2A gene in specific brain regions of Alzheimer's disease patients. Based on this finding, this invention provides a method for diagnosing or prognosticating Alzheimer's disease in a subject, or for determining whether a subject is at increased risk of developing Alzheimer's disease. Furthermore, this invention provides therapeutic and prophylactic methods for treating or preventing Alzheimer's disease and related neurodegenerative disorders using a voltage-gated ion channel gene, in particular the SCN2A gene. A method of screening for modulating agents of neurodegenerative diseases is also disclosed.

Figure 1: Identification of Genes Involved in Alzheimer's Disease Pathology

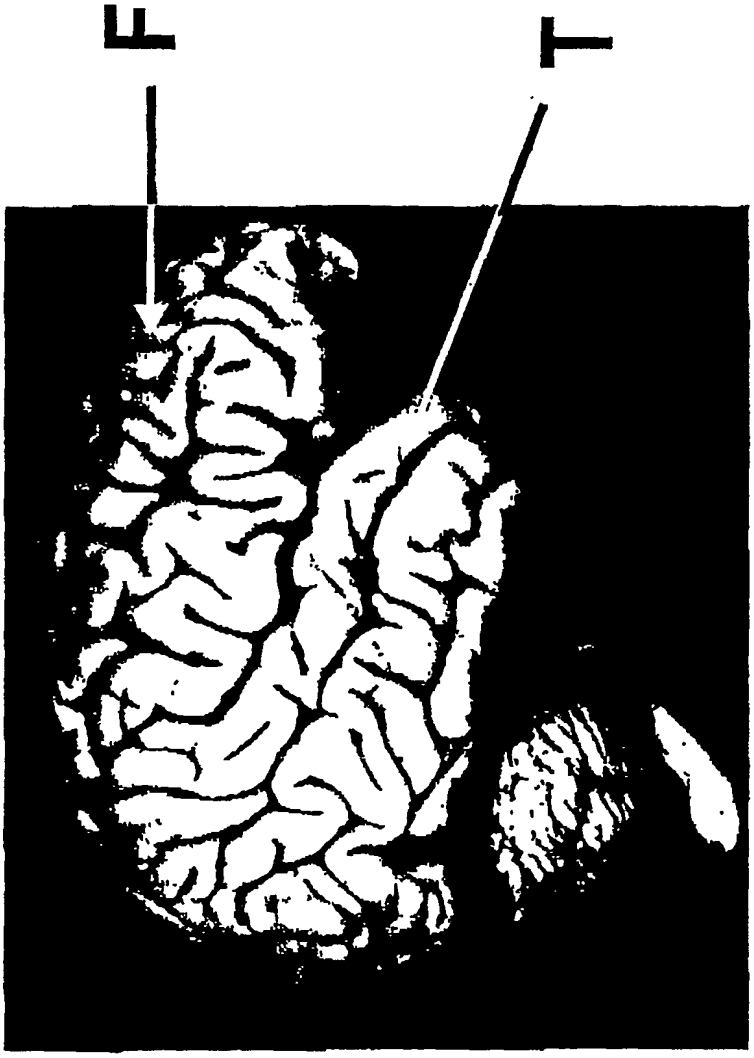

Figure 2: Identification of differentially expressed genes in a suppressive subtractive hybridization screen by dot blot analysis

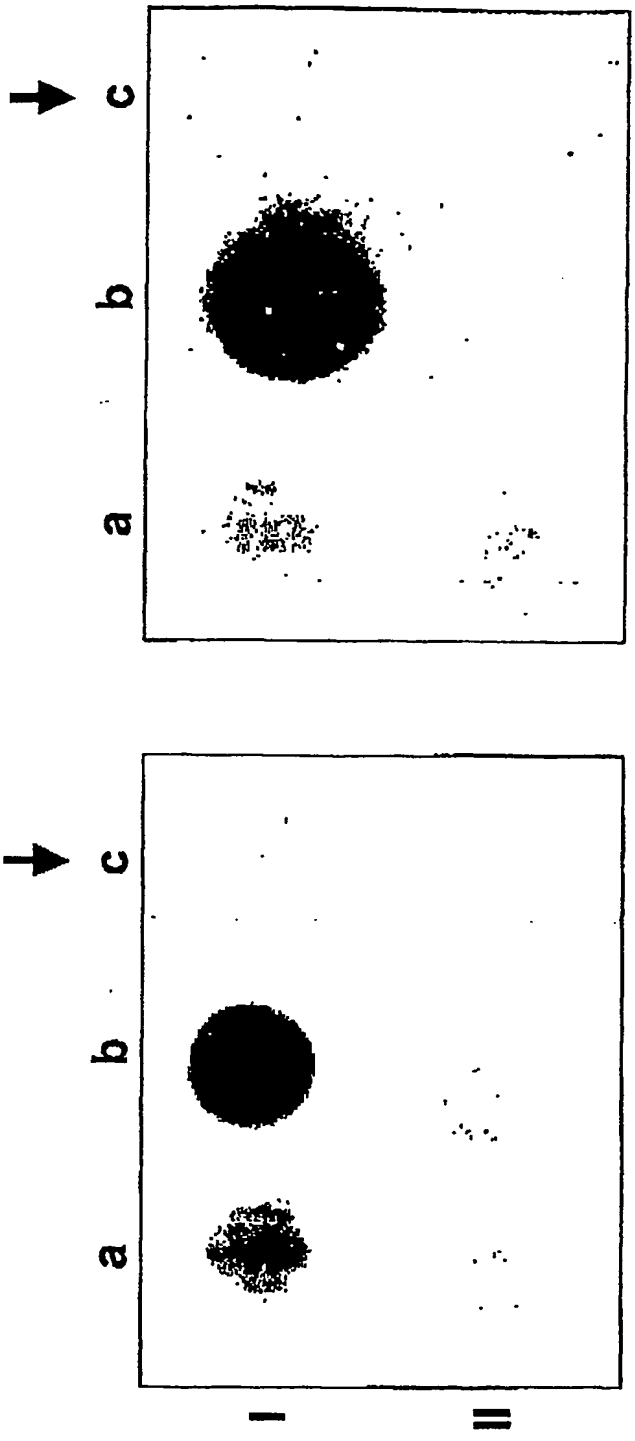

Figure 3: Verification of differential expression of SCN2A by quantitative RT-PCR

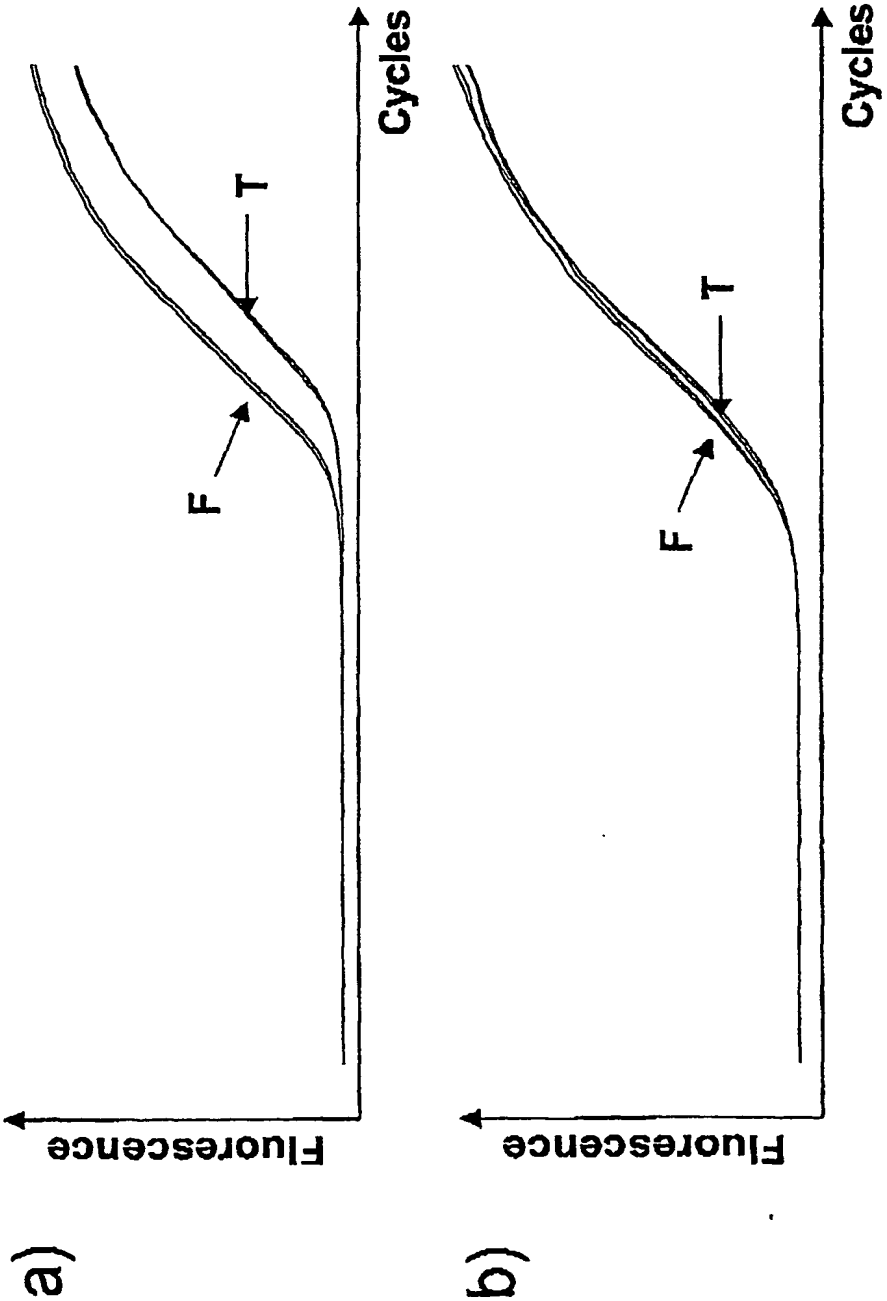

Figure 4: Nucleotide sequence of SEQ ID NO: 1

Length: 272 bp

```
  1  AATTAAGGTT GGAAGAATAA AAAGCAAGAA GCTCTTCCTT GTTTGCTGCA
 51  ACCTATTGCT TAATGACATG AAGAATGAGG TCTTGGTAGA ACAATTTGCT
101  TCACTTTACC ACTGATATAT GGCTTCCCAT ATTAGACTTC TGAACAGGGG
151  AAGGAATAAG ATACAGCAGC ATAGGCAAGA TAAACATGCA GCAGTGACAG
201  CTTCAAACTA TAATGGAACC AATTACATCA TATTACCTGT TGGAAGCTTG
251  CAAACTATAC TTACTGGGGT AC
```

Figure 5: Schematic alignment of SEQ ID NO: 1 with sodium channel type II alpha subunit (Accession No. AF327246)

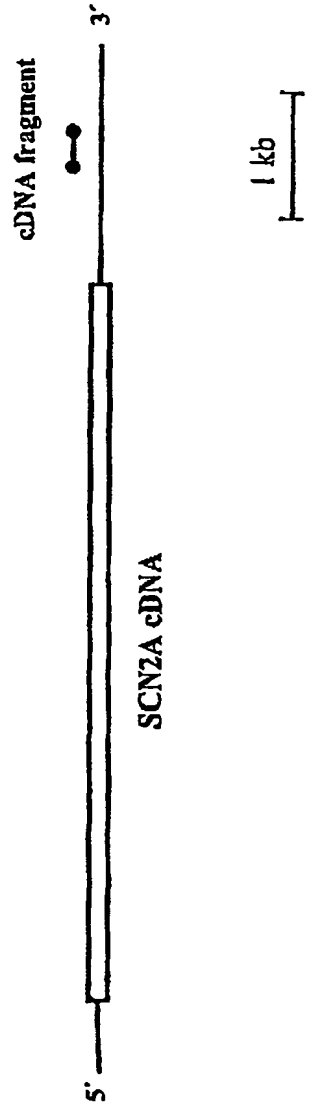

Figure 6: Alignment of SEQ ID NO: 1 to sodium channel type II alpha subunit mRNA (Accession No. AF327246)

Length: 272 bp
Percent Identity: 98.897

```
 272 GTACCCCAGTAAGTATAGTTTGCAAGCTTCCAACAGGTAATATGATGTAA 223
     ||||| ||||||||||||||||||||||| ||||||||||||||||||||||
2598 GTACCACAGTAAGTATAGTTTGCAAGCTTTCAACAGGTAATATGATGTAA 2647

 222 TTGGTTCCATTATAGTTTGAAGCTGTCACTGCTGCATGTTTATCTTGCCT 173
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2648 TTGGTTCCATTATAGTTTGAAGCTGTCACTGCTGCATGTTTATCTTGCCT 2697

 172 ATGCTGCTGTATCTTATTCCTTCCCCTGTTCAGAAGTCTAATATGGGAAG 123
     ||||||||||||||||||||||||| ||||||||||||||||||||||||
2698 ATGCTGCTGTATCTTATTCCTTCCACTGTTCAGAAGTCTAATATGGGAAG 2747

 122 CCATATATCAGTGGTAAAGTGAAGCAAATTGTTCTACCAAGACCTCATTC 73
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2748 CCATATATCAGTGGTAAAGTGAAGCAAATTGTTCTACCAAGACCTCATTC 2797

  72 TTCATGTCATTAAGCAATAGGTTGCAGCAAACAAGGAAGAGCTTCTTGCT 23
     ||||||||||||||||||||||||||||||||||||||||||||||||||||
2798 TTCATGTCATTAAGCAATAGGTTGCAGCAAACAAGGAAGAGCTTCTTGCT 2847

  22 TTTTATTCTTCCAACCTTAATT 1
     |||||||||||||||||||||||
2848 TTTTATTCTTCCAACCTTAATT 2869
```

Table 1:

sample	Δ (fold)
patient 1	2.93
patient 2	2.31
patient 3	3.16
patient 4	2.69
patient 5	2.44
patient 6	2.17
control 1	1.17
control 2	1.37
control 3	1.11
control 4	1.07
control 5	0.52
control 6	0.54

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